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(54) SALMON-DERIVED CHONDROITIN SULFURIC ACID

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a new chondroitin sulfuric acid which can be prepared massively at a lower cost and which can be used for a variety of useful applications and its preparation method.
SOLUTION: The chondroitin sulfuric acid comprises a disaccharide unit containing non-sulfated N-acetyl-D-galactosamine: (11.0±3.3)%, a disaccharide unit containing 6-sulfated N-acetyl-D-galactosamine: (52.8±15.8)%, a disaccharide unit containing 4-sulfated N-acetyl-D-galactosamine: (28.4±-8.5)% and a disaccharide unit containing 4,6-disulfated N-acetyl-D-galactosamine: (7.8±2.3)%. The preparation method of the chondroitin sulfuric acid comprises cryogenic- grinding of nasal cartilage of salmon, degreasing the same, treating the same with an alkali and pronase, centrifugation of the resulting digestive liquor and precipitation in ethanol. The method may further comprises treatment of the resulting precipitate with a cation-exchange resin.

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CLAIMS

[Claim(s)]
[Claim 1] The disaccharide unit containing non-sulfurating N-acetyl-D-galactosamine : (11.0**3.3) %, The disaccharide unit which contains 1 sulfation N-acetyl-D-galactosamine the 6th place of C : (52.8**15.8) %, The disaccharide unit which contains 1 sulfation N-acetyl-D-galactosamine the 4th place of C: (28.4**8.5) Chondroitin sulfate which changes including % and disaccharide unit:(7.8**2.3) % which contains disulfuric acid-ized N-acetyl-D-galactosamine C4 and the 6th place of C.
[Claim 2] Chondroitin sulfate according to claim 1 which has the structure with which the disaccharide unit containing non-sulfurating N-acetyl-D-galactosamine, the disaccharide unit which contains 1 sulfation N-acetyl-D-galactosamine the 6th place of C, the disaccharide unit which contains 1 sulfation N-acetyl-D-galactosamine the 4th place of C, and the disaccharide unit which contains disulfuric acid-ized N-acetyl-D-galactosamine C4 and the 6th place of C were located in a line at random.
[Claim 3] Chondroitin sulfate according to claim 1 obtained from the cartilagines nasi of a salmon.
[Claim 4] The manufacture approach of the chondroitin sulfate according to claim 1 which carries out low temperature grinding of the cartilagines nasi of a salmon, processes by alkali and pronase and is characterized by carrying out after [centrifugal separation] ethanol precipitate of the obtained digestive juices after degreasing.
[Claim 5] Furthermore, the manufacture approach of chondroitin sulfate according to claim 4 of processing the obtained precipitate by the cation exchange resin.
[Claim 6] The anti-inflammatory agent which comes to contain chondroitin sulfate according to claim 1.
[Claim 7] The moisturizer which comes to contain chondroitin sulfate according to claim 1.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the new chondroitin sulfate it is expected in the field of drugs, cosmetics, a food additive, etc. that various applications are.
[0002]
[Description of the Prior Art] Chondroitin sulfate is acid mucopolysaccharide contained in the connective tissue of an animal. This consists of D-glucuronic acid and the disaccharide repeat structure of the sulfurated N-acetyl galactosamine, and a variety of isomers exist by sulfation of the hydroxyl group of configuration sugar. The part in which it is sulfurated and deals is the hydroxyl group of the 4th place and the 6th place of the hydroxyl group of the 2nd place and the 3rd place of glucuronic acid or the hydroxyl group of the 2nd place of iduronic acid, and N-acetyl galactosamine. A chondroitin sulfate chain is the polysaccharide of the shape of a straight chain of molecular weight 104-105, and exists as proteoglycan which carried out covalent bond to core protein. There are few to which the chondroitin sulfate chain which exists naturally generally changes only from the repeat of one kind of sulfation disaccharide, and they usually contain sulfation of various classes, or a non-sulfurating disaccharide at a different rate. Chondroitin sulfate is most discovered early among acid mucopolysaccharide. Fischer and Boedeker will dissociate from a cartilage in 1886, and it is chondroit at first. It was named acid. Then, it turns out that it has a sulfate and came to be referred to as chondroitinsulfate. It was shown clearly by Meyer and others in further 1951 that A and C existed in chondroitin sulfate in a cartilage with three kind (A, B, C). As proteoglycan with a chondroitin sulfate chain, although there are AGURIKAN, bar SHIKAN, decorin, etc., these functions also have many unknown things. However, since the activity which controls the cartilaginous tissue function of AGURIKAN, and the anti-cell adhesion activity of bar SHIKAN disappear thoroughly by chondroitinase processing, a chondroitin sulfate chain is considered to bear those activity. Furthermore, although chondroitin sulfate proteoglycan was isolated as the neurotrophic factor to a retina nerve cell, and a neural spine extension factor, such activity also disappeared by chondroitinase processing. Recently, there is also a report that the disaccharide of the chondroitin sulfate origin suppresses activation of a spontaneous killer cell. Moreover, it was known from the appearance of a human immunodeficiency virus (HIV) or before that sulfation polysaccharides, such as chondroitin sulfate, a carrageenan, and a sulfation dextran, will check the infection to the cell of many viruses, such as an influenza virus and a herpes simplex virus. Thus, since various bioactive and physical properties are accepted in chondroitin sulfate, drugs, such as an anti-inflammatory agent, are begun, and it is used for cosmetics or the eye lotion as food additives, such as a gelling agent and a gelling agent, as a moisturizer, and can see in the unexpectedly large range in everyday life. Moreover, utility value potential as drugs of various objects etc. is expected from the property besides the application usual [these].
[0003]
[Problem(s) to be Solved by the Invention] The chondroitin sulfate used for current medical care etc. is chondroitin sulfate C (ChS-C, chondroitin 6 sulfuric acid) extracted from the pterygium of the chondroitin sulfate A (ChS-A, chondroitin 4 sulfuric acid) extracted from the whale cartilage, and a shark. Recently, although specific gravity is moving from ChS-A to ChS-C by the moratorium on whale hunting, also in the pterygium of a shark, the price is rising as foods of Chinese food. Therefore, the raw material and approach of acquiring chondroitin sulfate to a large quantity are searched for more cheaply. therefore, the object of this invention -- more -- cheap -- and a large quantity -- acquirable -- in addition -- and it is in the new chondroitin sulfate which can expect various useful applications, and offering the acquisition approach.
[0004]

[Means for Solving the Problem] The disaccharide unit in which this invention contains non-sulfurating N-acetyl-D-galactosamine (It abbreviates to un-sulfurating [GalNAc] hereafter.) :(11.0**3.3) %, the disaccharide unit which contains 1 sulfation N-acetyl-D-galactosamine the 6th place of C (it abbreviates to the 1 sulfation GalNAc the 6th place of C hereafter.) :(52.8**15.8) %, the disaccharide unit which contains 1 sulfation N-acetyl-D-galactosamine the 4th place of C (it abbreviates to the 1 sulfation GalNAc the 4th place of C hereafter.) : (28.4**8.5) % and the disaccharide unit which contains disulfuric acid-ized N-acetyl-D-galactosamine C4 and the 6th place of C (It abbreviates to disulfuric acid-ized GalNAc C4 and the 6th place of C hereafter.) : (7.8**2.3) -- the chondroitin sulfate which changes including % -- and Carry out low temperature grinding of the cartilagines nasi of a salmon, and after degreasing, it processes by alkali and pronase. It is related with the approach of processing the obtained precipitate by the cation exchange resin further in the acquisition approach of this chondroitin sulfate characterized by carrying out after [centrifugal separation] ethanol precipitate of the obtained digestive juices, and a list, and manufacturing this chondroitin sulfate in them. Moreover, this invention relates to the anti-inflammatory agent and moisturizer which come to contain this chondroitin sulfate.

[0005] The chondroitin sulfate of this invention is obtained from the cartilagines nasi of a salmon by the large quantity. The outline of the approach concerned is as follows. That is, let what separated only the cartilagines nasi from the head discharged in the processing process of a salmon except for epidermis, the bone, the meat grain, etc. first, and was ground at -120 degrees C be a raw material. By carrying out low temperature grinding, the denaturation and oxidation of the matter by generating of grinding heat can be prevented. Moreover, in ordinary temperature grinding, pulverizing to impossible size is attained and grain size is also arranged. Acid mucopolysaccharide is extracted from this cartilage powder, this is processed with proton exchange resin, and a chondroitin sulfate fraction is obtained. operating instructions etc. -- a whale and a shark -- it can refer to it of the chondroitin sulfate extraction method from a cartilage.

[0006] It comes to be below a profile when the period until it manufactures purification chondroitin sulfate from cartilage powder is described in more detail. That is, first, cartilage powder is degreased with organic solvents, such as an acetone, and this is processed in alkali water solutions, such as for example, a caustic-alkali-of-sodium water solution, and subsequently, after neutralizing, it digests by pronases, such as AKUCHINAZEE. Next, ethanol is added and precipitate is produced, after carrying out centrifugal separation of these digestive juices and making pH into acidity with an acetic acid etc. The produced precipitate is isolated preparatively by centrifugal separation actuation, and after washing by ethanol, reduced pressure drying of this is carried out. After dissolving the obtained acid mucopolysaccharide in a small amount of deionized water and processing this with the cation exchange resin of for example, DOWEX50WX2 grade, effluent is neutralized and this is dialyzed in deionized water. The obtained solution is condensed, and if it freeze-dries after filtering with a membrane filter etc., the refined material of chondroitin sulfate will be obtained.

[0007] The abundance ratio of the disaccharide which constitutes the chondroitin sulfate of this invention usually : [Non-Sulfurating / GalNAc / (%)] 11.0**3.3 1 sulfation GalNAc (%):52.8**15.8 the 6th place of C 1 sulfation GalNAc (%):28.4**8.5 the 4th place of C C4 and the 6th place of C -- disulfuric-acid-izing -- preferably, although it is GalNAc(%):7.8**2.3 : [Non-Sulfurating / GalNAc / (%)] 11.0**2.2 the 6th place of C -- 1 -- sulfation GalNAc(%):52.8**10.6 the 4th place of C -- 1 -- sulfation GalNAc(%):28.4**5.7 C4 and the 6th place of C -- disulfuric-acid-izing -- it is GalNAc(%):7.8**1.6.

[0008] As for the thing of such a rate, the abundance ratio of the disaccharide which constitutes chondroitin sulfate is not known until now. the shark which disulfuric acid-ized GalNAc has C4 and the 6th place of C as 1% or less, and is incidentally known for the chondroitin sulfate of the whale cartilage origin known from the former from the former similarly while there is much 1 sulfation GalNAc the 4th place of C as about 70% -- although there is much 1 sulfation GalNAc the 6th place of C at the chondroitin sulfate of the cartilage origin as about 70%, there is little 1 sulfation GalNAc the 4th place of C as about ten%. few]

[0009] Moreover, the chondroitin sulfate of this invention has structure with sulfuric-acid radical distribution more nearly random than the conventional thing. That is, it has the structure with which the disaccharide unit including un-sulfurating [GalNAc], the disaccharide unit which includes the 1 sulfation GalNAc the 6th place of C, the disaccharide unit which includes the 1 sulfation GalNAc the 4th place of C, and the disaccharide unit which contains disulfuric acid-ized GalNAc C4 and the 6th place of C were located in a line at random.

[0010] Like the conventional thing, the chondroitin sulfate of this invention begins drugs, such as an anti-inflammatory agent, and can use them for cosmetics or the eye lotion as food additives, such as a gelling agent and a gelling agent, as a moisturizer again. Furthermore, as future expansion, since physiological viscosity of chondroitin sulfate is high, it can consider the effectiveness of making the partial residence time of the drugs

extending with the blend with combination drugs. Moreover, since stabilization of a cornea collagenous fiber is promoted and it is reported that it is effective in functional maintenance of the organization of an eye, the application as a highly efficient nature skin substitute is also considered with the blend with the collagen extracted from salmon skin, oxhide, etc. Since physiology and not only pharmacological activity but the property as a polyelectrolyte is shown, industrial application is also still more possible. the chondroitin sulfate of this invention -- the chondroitin sulfate of the whale origin, and a shark -- since in-between structure with the chondroitin sulfate of the origin is taken, possibility of being applicable to the comparatively large range is expectable.

[0011]

[Example] Although an example is given to below and this invention is further explained to it at a detail, this invention is not limited at all by these examples.

[0012] Except for epidermis, the bone, the meat grain, etc., only the cartilagines nasi was separated from the head discharged in the processing process of the cleaning salmon of an example 1 (1) cartilage, and what was ground at -120 degrees C under liquid nitrogen was used as the raw material.

1) About 100mg cartilage powder was put into the 2000ml Erlenmeyer flask, acetone 700ml was added, and it stirred for 10 minutes.

2) It was left for 5 minutes and the supernatant was removed.

3) Actuation of 1-2 was repeated further 3 times.

4) The precipitate which remained was dried with the vacuum desiccator.

5) The obtained sample was saved at -30 degrees C.

(2) It is 0.2M in 5g of degreased [alkali treatment 1] cartilage powder. It dissolved in NaOH80ml.

2) It stirred in the water bath of 37 degree C for 3 hours.

3) The acetic acid neutralized to pH7.0.

(3) Pronase digestive 10.2M 10ml (pH7.8) of Tris-HCl buffer solutions was added.

2) Calcium acetate was added so that it might be set to final concentration 0.02M.

3) Methanol 5ml was added for preservation from decay.

4) AKUCHINAZE E50mg was added.

5) In the water bath of 37 degree C, it stirred slowly for 48 hours.

[0013] (4) Centrifugal separation of the ethanol precipitate 1 digestive juices was carried out at 4 degrees C for [10,000rpmx] 30 minutes.

2) Suction filtration of the supernatant was carried out using 0.45-micrometer membrane filter.

3) Calcium acetate equivalent to 5% was added to filtrate.

4) The acetic acid adjusted to pH4.5.

5) In addition to the ethanol of the amount of 2 double, it was left for 48 hours.

(5) Centrifugal separation of the washing / desiccation 1 ethanol liquid of precipitate was carried out at 4 degrees C for [7,000rpmx] 30 minutes.

2) Precipitate was collected, ethanol 300ml was added 80%, and it stirred slowly for 12 hours.

3) Centrifugal separation was carried out at 4 degrees C for [10,000rpmx] 30 minutes.

4) Actuation of 2-3 was repeated.

5) Ethanol 200ml was added to precipitate 100%, and it stirred slowly for 6 hours.

6) Centrifugal separation was carried out at 4 degrees C for [10,000rpmx] 30 minutes.

7) The obtained precipitate (acid mucopolysaccharide) was dried with the vacuum desiccator. (The appearance yield from cartilage [degreased] powder: 44.0%)

[0014] (6) 100ml of purification (6-1) pretreatment 1DOWEX 50WX2 cation exchange resin of chondroitin sulfate was stirred in 3N HCl for 2 hours, and it was stirred in 2N NaOH after rinsing for 2 hours.

2) It rinsed, after repeating the above-mentioned actuation 3 times.

3) Absorbent cotton was put in the bottom of a 2.5x40cm column, and resin was packed so that air might not enter.

(6-2) The obtained acid mucopolysaccharide which was obtained by the DOWEX 50Wx2 cation-exchange-resin processing 1 above (5) was dissolved in very a small amount of deionized water.

2) 1 was passed in the column and it was left for 20 minutes.

3) 400ml (about 4 times of the resin volume) deionized water was passed in the column.

4) Effluent was immediately neutralized by 1N NaOH.

(6-3) Purification 1 neutralization liquid was dialyzed for three days in deionized water.

2) It condensed to about 20ml in the evaporator.
3) It freeze-dried after filtration with the 0.22m membrane filter, and considered as the desiccation preparation. (The appearance yield from cartilage [degreased] powder: 24.0%)
[0015] The analysis result and consideration about the chondroitin sulfate (it is hereafter written as ChS-S.) of this invention obtained on the [analysis result] are shown below. in addition -- if in charge of these component analyses, structural analysis, etc. -- the quantum of aminosugar and uronic acid -- Morgan-Elson -- law and Bitter-Muir -- law was used. whenever [moreover, / permutation / of a sulfuric-acid radical] -- Rhodizonate -- law and elemental analysis performed. The molecular weight of ChS-S and purity assay used suitably GPC, FT-IR, and cellulose-acetate-membrane electrophoresis. Structural analysis is 13 C-NMR. (100MHz) It reaches. 1 H-NMR (400MHz) was used. Moreover, HPLC of the partial saturation disaccharide obtained by two-step lyase decomposition by Chondroitinase ABC and Chondroitinase AC 2 of ChS-S and 2-dimensional NMR analysis (COSY1 H-NMR hydrogen nucleus shift correlation etc.) performed decision of a sulfuric-acid permutation location, and its distribution. As a contrast sample, the commercial chondroitin 4-sulfuric acid (ChS-A, whale cartilage origin) and the chondroitin 6-sulfuric acid (shark ChS-C, cartilage origin) (all are the Seikagaku make) were used.
[0016] (1) the chondroitin sulfate content (purity) which the uronic acid contained in quantum each sample solution of uronic acid reached comparatively, and was calculated from this value -- a table 1 -- moreover, the processing (it abbreviates to the proton message exchange hereafter.) by cation exchange resin (DOWEX 50WX2) showed the effect which it has on purity and yield in a table 2, respectively. In addition, the uronic acid content of standard chondroitin sulfate is 37%. These results showed that the chondroitin sulfate of a high grade could be dramatically obtained by this method. Moreover, it is before and after the proton message exchange, and purity is increasing substantially. It is thought that other acid mucopolysaccharide, such as hyaluronic acid and dermatan sulfate, was thoroughly removable from this with the proton message exchange.
[0017]
[A table 1]

ウロン酸含量及び純度

	精製前ChS-S	ChS-S	ChS-A	ChS-C
ウロン酸（%）	25.50	36.49	35.63	36.88
純度（%）	68.92	98.62	96.30	99.68

[0018]
[A table 2]

各操作毎の本発明のコンドロイチン硫酸の収率

	見掛歩留（%）	純度（%）	収率（%）
脱脂軟骨	100	・・・	・・・
エタノール沈殿	44.0	68.9	30.3
プロトン交換処理	24.0	98.6	23.7

[0019] (2) The N-acetyl galactosamine (GalNAc) content of aminosugar analysis each sample solution was shown in a table 3. Moreover, although N-acetyl glucosamine was detected about 0.3% from the acid mucopolysaccharide before the proton message exchange, it was not checked at all by ChS-S, ChS-A, and ChS-C. Other aminosugar was contained in neither. From this, it has checked that the aminosugar which constitutes chondroitin sulfate is only N-acetyl galactosamine, and that other aminosugar was thoroughly removable with the proton message exchange.
[0020]
[A table 3]

コンドロイチン硫酸のN-アセチルガラクトサミン含量

	精製前ChS-S	ChS-S	ChS-A	ChS-C
GalNAc（%）	25.46	32.62	30.70	26.73

[0021] (3) Whenever [weight ratio / of the carbon contained in elemental-analysis each sample, hydrogen, nitrogen, oxygen, and sulfur / and sulfation] was shown in a table 4 and a table 5, respectively. From a table 4, it

was confirmed that the presentation with any same sample is shown. whenever [from a table 5 / sulfation] -- a shark -- cartilage origin chondroitin sulfate was the highest and the result [chondroitin sulfate / of this invention / / else] of being a little low was obtained.

[0022]

[A table 4]

コンドロイチン硫酸の組成分析結果

	C (%)	H (%)	N (%)	O (%)	S (%)
ChS-S	39.86	5.48	3.25	45.91	5.50
ChS-A	39.96	5.45	3.30	45.40	5.89
ChS-C	40.97	5.62	3.15	43.98	6.28

[0023]

[A table 5]

コンドロイチン硫酸1分子当たりの硫酸基含量

ChS-S	ChS-A	ChS-C
0.72	0.77	0.80

[0024] (4) The ratio of the sulfur computed from the sulfuric-acid radical contained in sulfuric-acid radical quantum each sample and here was shown in a table 6. The sulfuric-acid radical content had a little much ChS-C, and this result showed that ChS-S and ChS-A were almost the same. Moreover, this was in agreement also with the result of the elemental analysis described above (3).

[0025]

[A table 6]

各コンドロイチン硫酸の硫酸及び硫黄含量

	硫酸基 (%)	硫黄 (%)
ChS-S	18.62	6.15
ChS-A	18.52	6.15
ChS-C	21.74	7.17

[0026] (5) The average molecular weight and molecular weight distribution of determination-of-molecular-weight each sample were shown in a table 7. The average molecular weight of the chondroitin sulfate of this invention was 173,000. Although this value is [/ else] a little large, molecular weight distribution are mostly in agreement. Generally it is known that the molecular weight of chondroitin sulfate will take the value of 50,000-300,000 by the extract approach. The molecular weight distribution of the chondroitin sulfate obtained this time were also matches at this.

[0027]

[A table 7]

コンドロイチン硫酸の平均分子量及び分子量分布

	ChS-S	ChS-A	ChS-C
平均分子量	173,000	151,000	108,000
分子量分布	5-30×10⁴	5-30×10⁴	5-30×10⁴

[0028] (6) The abundance ratio of the disaccharide which constitutes each chondroitin sulfate which carried out fractionation of the chondroitin sulfate which used together the chondroitin dialytic ferment of two kinds of HPLC, and was understood by the enzyme using HPLC, and was obtained by carrying out a quantum is shown in a table 8. Moreover, whenever [sulfation / of the chondroitin sulfate for which it asked from here] (the number of the sulfuric-acid radicals per GalNAcl molecule) was shown in a table 9. the chondroitin sulfate of a table 8 to this invention -- the chondroitin sulfate of the whale origin, and a shark -- it turned out that in-between structure with the chondroitin sulfate of the origin is taken. So, it is thought that the chondroitin sulfate of this

invention will be applicable to the comparatively large range.

[0029]

[A table 8]

コンドロイチン硫酸の構成不飽和二糖

	ΔDi-0S	ΔDi-6S	ΔDi-4S	ΔDi-di4,6S
ChS-S (%)	11.0	52.8	28.4	7.8
ChS-A (%)	3.6	25.3	70.2	0.6
ChS-C (%)	7.7	71.2	13.2	7.6

ΔDi-0S : 非硫酸化GalNAc

ΔDi-6S : C6位一硫酸化GalNAc

ΔDi-6S : C4位一硫酸化GalNAc

ΔDi-di4,6S : C4,C6位二硫酸化GalNAc

[0030]

[A table 9]

各々のコンドロイチン硫酸の硫酸化度

	ChS-S	ChS-A	ChS-C
硫酸化度	0.968	0.973	1.002

[0031]

[Effect of the Invention] the chondroitin sulfate of the whale origin by which the chondroitin sulfate of this invention is known from the former, and a shark, in order to take in-between structure with the chondroitin sulfate of the origin Possibility of being applicable to the comparatively large range is high, and begins drugs, such as an anti-inflammatory agent, like the conventional thing. As a moisturizer that it can use for cosmetics or the eye lotion as food additives, such as a gelling agent and a gelling agent, again from the first From average molecular weight being larger than the conventional thing, it is thought that physiological viscosity is higher than the conventional thing, and the effectiveness of making the partial residence time of the drugs extending with the blend with combination drugs can be expected. Moreover, as the latest report of chondroitin sulfate, stabilization of a cornea collagenous fiber is promoted and the application as a highly efficient nature skin substitute can also expect a report of a purport effective in functional maintenance of the organization of an eye from a certain thing with the blend with the collagen extracted from salmon skin, oxhide, etc. Furthermore, since physiology and not only pharmacological activity but the property as a polyelectrolyte is shown, industrial application is also expectable.

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TECHNICAL FIELD

[Field of the Invention] This invention relates to the new chondroitin sulfate it is expected in the field of drugs, cosmetics, a food additive, etc. that various applications are.

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PRIOR ART

[Description of the Prior Art] Chondroitin sulfate is acid mucopolysaccharide contained in the connective tissue of an animal. This consists of D-glucuronic acid and the disaccharide repeat structure of the sulfurated N-acetyl galactosamine, and a variety of isomers exist by sulfation of the hydroxyl group of configuration sugar. The part in which it is sulfurated and deals is the hydroxyl group of the 4th place and the 6th place of the hydroxyl group of the 2nd place and the 3rd place of glucuronic acid or the hydroxyl group of the 2nd place of iduronic acid, and N-acetyl galactosamine. A chondroitin sulfate chain is the polysaccharide of the shape of a straight chain of molecular weight 104-105, and exists as proteoglycan which carried out covalent bond to core protein. There are few to which the chondroitin sulfate chain which exists naturally generally changes only from the repeat of one kind of sulfation disaccharide, and they usually contain sulfation of various classes, or a non-sulfurating disaccharide at a different rate. Chondroitin sulfate is most discovered early among acid mucopolysaccharide. Fischer and Boedeker will dissociate from a cartilage in 1886, and it is chondroit at first. It was named acid. Then, it turns out that it has a sulfate and came to be referred to as chondroitinsulfate. It was shown clearly by Meyer and others in further 1951 that A and C existed in chondroitin sulfate in a cartilage with three kind (A, B, C). As proteoglycan with a chondroitin sulfate chain, although there are AGURIKAN, bar SHIKAN, decorin, etc., these functions also have many unknown things. However, since the activity which controls the cartilaginous tissue function of AGURIKAN, and the anti-cell adhesion activity of bar SHIKAN disappear thoroughly by chondroitinase processing, a chondroitin sulfate chain is considered to bear those activity. Furthermore, although chondroitin sulfate proteoglycan was isolated as the neurotrophic factor to a retina nerve cell, and a neural spine extension factor, such activity also disappeared by chondroitinase processing. Recently, there is also a report that the disaccharide of the chondroitin sulfate origin suppresses activation of a spontaneous killer cell. Moreover, it was known from the appearance of a human immunodeficiency virus (HIV) or before that sulfation polysaccharides, such as chondroitin sulfate, a carrageenan, and a sulfation dextran, will check the infection to the cell of many viruses, such as an influenza virus and a herpes simplex virus. Thus, since various bioactive and physical properties are accepted in chondroitin sulfate, drugs, such as an anti-inflammatory agent, are begun, and it is used for cosmetics or the eye lotion as food additives, such as a gelling agent and a gelling agent, as a moisturizer, and can see in the unexpectedly large range in everyday life. Moreover, utility value potential as drugs of various objects etc. is expected from the property besides the application usual [these].

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EFFECT OF THE INVENTION

[Effect of the Invention] the chondroitin sulfate of the whale origin by which the chondroitin sulfate of this invention is known from the former, and a shark, in order to take in-between structure with the chondroitin sulfate of the origin Possibility of being applicable to the comparatively large range is high, and begins drugs, such as an anti-inflammatory agent, like the conventional thing. As a moisturizer that it can use for cosmetics or the eye lotion as food additives, such as a gelling agent and a gelling agent, again from the first From average molecular weight being larger than the conventional thing, it is thought that physiological viscosity is higher than the conventional thing, and the effectiveness of making the partial residence time of the drugs extending with the blend with combination drugs can be expected. Moreover, as the latest report of chondroitin sulfate, stabilization of a cornea collagenous fiber is promoted and the application as a highly efficient nature skin substitute can also expect a report of a purport effective in functional maintenance of the organization of an eye from a certain thing with the blend with the collagen extracted from salmon skin, oxhide, etc. Furthermore, since physiology and not only pharmacological activity but the property as a polyelectrolyte is shown, industrial application is also expectable.

[Translation done.]

* NOTICES *

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TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] The chondroitin sulfate used for current medical care etc. is chondroitin sulfate C (ChS-C, chondroitin 6 sulfuric acid) extracted from the pterygium of the chondroitin sulfate A (ChS-A, chondroitin 4 sulfuric acid) extracted from the whale cartilage, and a shark. Recently, although specific gravity is moving from ChS-A to ChS-C by the moratorium on whale hunting, also in the pterygium of a shark, the price is rising as foods of Chinese food. Therefore, the raw material and approach of acquiring chondroitin sulfate to a large quantity are searched for more cheaply. therefore, the object of this invention -- more -- cheap -- and a large quantity -- acquirable -- in addition -- and it is in the new chondroitin sulfate which can expect various useful applications, and offering the acquisition approach.

[Translation done.]

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MEANS

[Means for Solving the Problem] The disaccharide unit in which this invention contains non-sulfurating N-acetyl-D-galactosamine (It abbreviates to un-sulfurating [GalNAc] hereafter.) :(11.0**3.3) %, the disaccharide unit which contains 1 sulfation N-acetyl-D-galactosamine the 6th place of C (it abbreviates to the 1 sulfation GalNAc the 6th place of C hereafter.) :(52.8**15.8) %, the disaccharide unit which contains 1 sulfation N-acetyl-D-galactosamine the 4th place of C (it abbreviates to the 1 sulfation GalNAc the 4th place of C hereafter.) : (28.4**8.5) % and the disaccharide unit which contains disulfuric acid-ized N-acetyl-D-galactosamine C4 and the 6th place of C (It abbreviates to disulfuric acid-ized GalNAc C4 and the 6th place of C hereafter.) : (7.8**2.3) -- the chondroitin sulfate which changes including % -- and Carry out low temperature grinding of the cartilagines nasi of a salmon, and after degreasing, it processes by alkali and pronase. It is related with the approach of processing the obtained precipitate by the cation exchange resin further in the acquisition approach of this chondroitin sulfate characterized by carrying out after [centrifugal separation] ethanol precipitate of the obtained digestive juices, and a list, and manufacturing this chondroitin sulfate in them. Moreover, this invention relates to the anti-inflammatory agent and moisturizer which come to contain this chondroitin sulfate.
[0005] The chondroitin sulfate of this invention is obtained from the cartilagines nasi of a salmon by the large quantity. The outline of the approach concerned is as follows. That is, let what separated only the cartilagines nasi from the head discharged in the processing process of a salmon except for epidermis, the bone, the meat grain, etc. first, and was ground at -120 degrees C be a raw material. By carrying out low temperature grinding, the denaturation and oxidation of the matter by generating of grinding heat can be prevented. Moreover, in ordinary temperature grinding, pulverizing to impossible size is attained and grain size is also arranged. Acid mucopolysaccharide is extracted from this cartilage powder, this is processed with proton exchange resin, and a chondroitin sulfate fraction is obtained. operating instructions etc. -- a whale and a shark -- it can refer to it of the chondroitin sulfate extraction method from a cartilage.
[0006] It comes to be below a profile when the period until it manufactures purification chondroitin sulfate from cartilage powder is described in more detail. That is, first, cartilage powder is degreased with organic solvents, such as an acetone, and this is processed in alkali water solutions, such as for example, a caustic-alkali-of-sodium water solution, and subsequently, after neutralizing, it digests by pronases, such as AKUCHINAZEE. Next, ethanol is added and precipitate is produced, after carrying out centrifugal separation of these digestive juices and making pH into acidity with an acetic acid etc. The produced precipitate is isolated preparatively by centrifugal separation actuation, and after washing by ethanol, reduced pressure drying of this is carried out. After dissolving the obtained acid mucopolysaccharide in a small amount of deionized water and processing this with the cation exchange resin of for example, DOWEX50WX2 grade, effluent is neutralized and this is dialyzed in deionized water. The obtained solution is condensed, and if it freeze-dries after filtering with a membrane filter etc., the refined material of chondroitin sulfate will be obtained.
[0007] The abundance ratio of the disaccharide which constitutes the chondroitin sulfate of this invention usually : [Non-Sulfurating / GalNAc / (%)] 11.0**3.3 1 sulfation GalNAc (%):52.8**15.8 the 6th place of C 1 sulfation GalNAc (%):28.4**8.5 the 4th place of C C4 and the 6th place of C -- disulfuric-acid-izing -- preferably, although it is GalNAc(%):7.8**2.3 : [Non-Sulfurating / GalNAc / (%)] 11.0**2.2 the 6th place of C -- 1 -- sulfation GalNAc(%):52.8**10.6 the 4th place of C -- 1 -- sulfation GalNAc(%):28.4**5.7 C4 and the 6th place of C -- disulfuric-acid-izing -- it is GalNAc(%):7.8**1.6.
[0008] As for the thing of such a rate, the abundance ratio of the disaccharide which constitutes chondroitin sulfate is not known until now. the shark which disulfuric acid-ized GalNAc has C4 and the 6th place of C as 1% or less, and is incidentally known for the chondroitin sulfate of the whale cartilage origin known from the former

from the former similarly while there is much 1 sulfation GalNAc the 4th place of C as about 70% -- although there is much 1 sulfation GalNAc the 6th place of C at the chondroitin sulfate of the cartilage origin as about 70%, there is little 1 sulfation GalNAc the 4th place of C as about ten%. few]

[0009] Moreover, the chondroitin sulfate of this invention has structure with sulfuric-acid radical distribution more nearly random than the conventional thing. That is, it has the structure with which the disaccharide unit including un-sulfurating [GalNAc], the disaccharide unit which includes the 1 sulfation GalNAc the 6th place of C, the disaccharide unit which includes the 1 sulfation GalNAc the 4th place of C, and the disaccharide unit which contains disulfuric acid-ized GalNAc C4 and the 6th place of C were located in a line at random.

[0010] Like the conventional thing, the chondroitin sulfate of this invention begins drugs, such as an anti-inflammatory agent, and can use them for cosmetics or the eye lotion as food additives, such as a gelling agent and a gelling agent, as a moisturizer again. Furthermore, as future expansion, since physiological viscosity of chondroitin sulfate is high, it can consider the effectiveness of making the partial residence time of the drugs extending with the blend with combination drugs. Moreover, since stabilization of a cornea collagenous fiber is promoted and it is reported that it is effective in functional maintenance of the organization of an eye, the application as a highly efficient nature skin substitute is also considered with the blend with the collagen extracted from salmon skin, oxhide, etc. Since physiology and not only pharmacological activity but the property as a polyelectrolyte is shown, industrial application is also still more possible. the chondroitin sulfate of this invention -- the chondroitin sulfate of the whale origin, and a shark -- since in-between structure with the chondroitin sulfate of the origin is taken, possibility of being applicable to the comparatively large range is expectable.

[Translation done.]

* NOTICES *

EXAMPLE

[Example] Although an example is given to below and this invention is further explained to it at a detail, this invention is not limited at all by these examples.
[0012] Except for epidermis, the bone, the meat grain, etc., only the cartilagines nasi was separated from the head discharged in the processing process of the cleaning salmon of an example 1 (1) cartilage, and what was ground at -120 degrees C under liquid nitrogen was used as the raw material.
1) About 100mg cartilage powder was put into the 2000ml Erlenmeyer flask, acetone 700ml was added, and it stirred for 10 minutes.
2) It was left for 5 minutes and the supernatant was removed.
3) Actuation of 1-2 was repeated further 3 times.
4) The precipitate which remained was dried with the vacuum desiccator.
5) The obtained sample was saved at -30 degrees C.
(2) It is 0.2M in 5g of degreased [alkali treatment 1] cartilage powder. It dissolved in NaOH80ml.
2) It stirred in the water bath of 37 degree C for 3 hours.
3) The acetic acid neutralized to pH7.0.
(3) Pronase digestive 10.2M 10ml (pH7.8) of Tris-HCl buffer solutions was added.
2) Calcium acetate was added so that it might be set to final concentration 0.02M.
3) Methanol 5ml was added for preservation from decay.
4) AKUCHINAZE E50mg was added.
5) In the water bath of 37 degree C, it stirred slowly for 48 hours.
[0013] (4) Centrifugal separation of the ethanol precipitate 1 digestive juices was carried out at 4 degrees C for [10,000rpmx] 30 minutes.
2) Suction filtration of the supernatant was carried out using 0.45-micrometer membrane filter.
3) Calcium acetate equivalent to 5% was added to filtrate.
4) The acetic acid adjusted to pH4.5.
5) In addition to the ethanol of the amount of 2 double, it was left for 48 hours.
(5) Centrifugal separation of the washing / desiccation 1 ethanol liquid of precipitate was carried out at 4 degrees C for [7,000rpmx] 30 minutes.
2) Precipitate was collected, ethanol 300ml was added 80%, and it stirred slowly for 12 hours.
3) Centrifugal separation was carried out at 4 degrees C for [10,000rpmx] 30 minutes.
4) Actuation of 2-3 was repeated.
5) Ethanol 200ml was added to precipitate 100%, and it stirred slowly for 6 hours.
6) Centrifugal separation was carried out at 4 degrees C for [10,000rpmx] 30 minutes.
7) The obtained precipitate (acid mucopolysaccharide) was dried with the vacuum desiccator. (The appearance yield from cartilage [degreased] powder: 44.0%)
[0014] (6) 100ml of purification (6-1) pretreatment 1DOWEX 50WX2 cation exchange resin of chondroitin sulfate was stirred in 3N HCl for 2 hours, and it was stirred in 2N NaOH after rinsing for 2 hours.
2) It rinsed, after repeating the above-mentioned actuation 3 times.
3) Absorbent cotton was put in the bottom of a 2.5x40cm column, and resin was packed so that air might not enter.
(6-2) The obtained acid mucopolysaccharide which was obtained by the DOWEX 50Wx2 cation-exchange-resin processing 1 above (5) was dissolved in very a small amount of deionized water.
2) 1 was passed in the column and it was left for 20 minutes.

3) 400ml (about 4 times of the resin volume) deionized water was passed in the column.
4) Effluent was immediately neutralized by 1N NaOH.
(6-3) Purification 1 neutralization liquid was dialyzed for three days in deionized water.
2) It condensed to about 20ml in the evaporator.
3) It freeze-dried after filtration with the 0.22m membrane filter, and considered as the desiccation preparation. (The appearance yield from cartilage [degreased] powder: 24.0%)
[0015] The analysis result and consideration about the chondroitin sulfate (it is hereafter written as ChS-S.) of this invention obtained on the [analysis result] are shown below. in addition -- if in charge of these component analyses, structural analysis, etc. -- the quantum of aminosugar and uronic acid -- Morgan-Elson -- law and Bitter-Muir -- law was used. whenever [moreover, / permutation / of a sulfuric-acid radical] -- Rhodizonate -- law and elemental analysis performed. The molecular weight of ChS-S and purity assay used suitably GPC, FT-IR, and cellulose-acetate-membrane electrophoresis. Structural analysis is 13 C-NMR. (100MHz) It reaches. 1 H-NMR (400MHz) was used. Moreover, HPLC of the partial saturation disaccharide obtained by two-step lyase decomposition by Chondroitinase ABC and Chondroitinase AC 2 of ChS-S and 2-dimensional NMR analysis (COSY1 H-NMR hydrogen nucleus shift correlation etc.) performed decision of a sulfuric-acid permutation location, and its distribution. As a contrast sample, the commercial chondroitin 4-sulfuric acid (ChS-A, whale cartilage origin) and the chondroitin 6-sulfuric acid (shark ChS-C, cartilage origin) (all are the Seikagaku make) were used.
[0016] (1) the chondroitin sulfate content (purity) which the uronic acid contained in quantum each sample solution of uronic acid reached comparatively, and was calculated from this value -- a table 1 -- moreover, the processing (it abbreviates to the proton message exchange hereafter.) by cation exchange resin (DOWEX 50WX2) showed the effect which it has on purity and yield in a table 2, respectively. In addition, the uronic acid content of standard chondroitin sulfate is 37%. These results showed that the chondroitin sulfate of a high grade could be dramatically obtained by this method. Moreover, it is before and after the proton message exchange, and purity is increasing substantially. It is thought that other acid mucopolysaccharide, such as hyaluronic acid and dermatan sulfate, was thoroughly removable from this with the proton message exchange.
[0017]
[A table 1]

ウロン酸含量及び純度

	精製前ChS-S	ChS-S	ChS-A	ChS-C
ウロン酸（%）	25.50	36.49	35.63	36.88
純度（%）	68.92	98.62	96.30	99.68

[0018]
[A table 2]

各操作毎の本発明のコンドロイチン硫酸の収率

	見掛歩留（%）	純度（%）	収率（%）
脱脂軟骨	100	・・・	・・・
エタノール沈殿	44.0	68.9	30.3
プロトン交換処理	24.0	98.6	23.7

[0019] (2) The N-acetyl galactosamine (GalNAc) content of aminosugar analysis each sample solution was shown in a table 3. Moreover, although N-acetyl glucosamine was detected about 0.3% from the acid mucopolysaccharide before the proton message exchange, it was not checked at all by ChS-S, ChS-A, and ChS-C. Other aminosugar was contained in neither. From this, it has checked that the aminosugar which constitutes chondroitin sulfate is only N-acetyl galactosamine, and that other aminosugar was thoroughly removable with the proton message exchange.
[0020]
[A table 3]

コンドロイチン硫酸のN-アセチルガラクトサミン含量

	精製前ChS-S	ChS-S	ChS-A	ChS-C
GalNAc（%）	25.46	32.62	30.70	26.73

[0021] (3) Whenever [weight ratio / of the carbon contained in elemental-analysis each sample, hydrogen, nitrogen, oxygen, and sulfur / and sulfation] was shown in a table 4 and a table 5, respectively. From a table 4, it was confirmed that the presentation with any same sample is shown. whenever [from a table 5 / sulfation] -- a shark -- cartilage origin chondroitin sulfate was the highest and the result [chondroitin sulfate / of this invention / / else] of being a little low was obtained.

[0022]

[A table 4]

コンドロイチン硫酸の組成分析結果

	C (%)	H (%)	N (%)	O (%)	S (%)
ChS-S	39.86	5.48	3.25	45.91	5.50
ChS-A	39.96	5.45	3.30	45.40	5.89
ChS-C	40.97	5.62	3.15	43.98	6.28

[0023]

[A table 5]

コンドロイチン硫酸1分子当たりの硫酸基含量

ChS-S	ChS-A	ChS-C
0.72	0.77	0.80

[0024] (4) The ratio of the sulfur computed from the sulfuric-acid radical contained in sulfuric-acid radical quantum each sample and here was shown in a table 6. The sulfuric-acid radical content had a little much ChS-C, and this result showed that ChS-S and ChS-A were almost the same. Moreover, this was in agreement also with the result of the elemental analysis described above (3).

[0025]

[A table 6]

各コンドロイチン硫酸の硫酸及び硫黄含量

	硫酸基 (%)	硫黄 (%)
ChS-S	18.62	6.15
ChS-A	18.52	6.15
ChS-C	21.74	7.17

[0026] (5) The average molecular weight and molecular weight distribution of determination-of-molecular-weight each sample were shown in a table 7. The average molecular weight of the chondroitin sulfate of this invention was 173,000. Although this value is [/ else] a little large, molecular weight distribution are mostly in agreement. Generally it is known that the molecular weight of chondroitin sulfate will take the value of 50,000-300,000 by the extract approach. The molecular weight distribution of the chondroitin sulfate obtained this time were also matches at this.

[0027]

[A table 7]

コンドロイチン硫酸の平均分子量及び分子量分布

	ChS-S	ChS-A	ChS-C
平均分子量	173,000	151,000	108,000
分子量分布	5-30×10⁴	5-30×10⁴	5-30×10⁴

[0028] (6) The abundance ratio of the disaccharide which constitutes each chondroitin sulfate which carried out fractionation of the chondroitin sulfate which used together the chondroitin dialytic ferment of two kinds of HPLC, and was understood by the enzyme using HPLC, and was obtained by carrying out a quantum is shown in a table 8. Moreover, whenever [sulfation / of the chondroitin sulfate for which it asked from here] (the number

of the sulfuric-acid radicals per GalNAcl molecule) was shown in a table 9. the chondroitin sulfate of a table 8 to this invention -- the chondroitin sulfate of the whale origin, and a shark -- it turned out that in-between structure with the chondroitin sulfate of the origin is taken. So, it is thought that the chondroitin sulfate of this invention will be applicable to the comparatively large range.

[0029]

[A table 8]

コンドロイチン硫酸の構成不飽和二糖

	ΔDi-0S	ΔDi-6S	ΔDi-4S	ΔDi-di4,6S
ChS-S (%)	11.0	52.8	28.4	7.8
ChS-A (%)	3.6	25.3	70.2	0.6
ChS-C (%)	7.7	71.2	13.2	7.6

ΔDi-0S : 非硫酸化GalNAc

ΔDi-6S : C6位一硫酸化GalNAc

ΔDi-6S : C4位一硫酸化GalNAc

ΔDi-di4,6S : C4,C6位二硫酸化GalNAc

[0030]

[A table 9]

各々のコンドロイチン硫酸の硫酸化度

	ChS-S	ChS-A	ChS-C
硫酸化度	0.968	0.973	1.002

[Translation done.]

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(54)【発明の名称】　鮭由来のコンドロイチン硫酸

(57)【要約】
【課題】　本発明は、より安価に、且つ大量に製造でき、尚かつ種々の有用な用途が期待できる新規なコンドロイチン硫酸と、その製造方法を提供することを目的とする。
【解決手段】　非硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンを含む二糖単位：（１１．０±３．３）％、Ｃ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンを含む二糖単位：（５２．８±１５．８）％、Ｃ４位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンを含む二糖単位：（２８．４±８．５）％、及びＣ４，Ｃ６位二硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンを含む二糖単位：（７．８±２．３）％を含んで成るコンドロイチン硫酸、及び、鮭の鼻軟骨を低温粉砕し、脱脂した後、アルカリ及びブロナーゼで処理し、得られた消化液を遠心分離後エタノール沈殿させることを特徴とする該コンドロイチン硫酸の製造方法、並びに、得られた沈殿を、更に、陽イオン交換樹脂で処理する該コンドロイチン硫酸の製造方法。

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【特許請求の範囲】

【請求項１】　非硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンを含む二糖単位：（１１．０±３．３）％、Ｃ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンを含む二糖単位：（５２．８±１５．８）％、Ｃ４位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンを含む二糖単位：（２８．４±８．５）％、及びＣ４，Ｃ６位二硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンを含む二糖単位：（７．８±２．３）％を含んで成るコンドロイチン硫酸。

【請求項２】　非硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンを含む二糖単位、Ｃ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンを含む二糖単位、Ｃ４位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンを含む二糖単位、及びＣ４，Ｃ６位二硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンを含む二糖単位がランダムに並んだ構造を有する請求項１に記載のコンドロイチン硫酸。

【請求項３】　鮭の鼻軟骨から得られた請求項１に記載のコンドロイチン硫酸。

【請求項４】　鮭の鼻軟骨を低温粉砕し、脱脂した後、アルカリ及びプロナーゼで処理し、得られた消化液を遠心分離後エタノール沈殿させることを特徴とする請求項１に記載のコンドロイチン硫酸の製造方法。

【請求項５】　更に、得られた沈殿を、陽イオン交換樹脂で処理する請求項４に記載のコンドロイチン硫酸の製造方法。

【請求項６】　請求項１に記載のコンドロイチン硫酸を含んでなる抗炎症剤。

【請求項７】　請求項１に記載のコンドロイチン硫酸を含んでなる保湿剤。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】本発明は、医薬品、化粧品、食品添加物等の分野において種々の用途が期待される新規なコンドロイチン硫酸に関する。

【０００２】

【従来の技術】コンドロイチン硫酸は、動物の結合組織に含まれる酸性ムコ多糖である。これは、Ｄ－グルクロン酸と、硫酸化されたＮ－アセチルガラクトサミンの二糖繰り返し構造から成り、構成糖の水酸基の硫酸化により多種多様な異性体が存在する。　硫酸化されうる部位はグルクロン酸の２位及び３位の水酸基、あるいはイズロン酸の２位の水酸基、及びＮ－アセチルガラクトサミンの４位及び６位の水酸基である。コンドロイチン硫酸鎖は、分子量10^4－10^5の直鎖状の多糖で、コア蛋白質に共有結合したプロテオグリカンとして存在する。一般に、天然に存在するコンドロイチン硫酸鎖は１種類の硫酸化二糖の繰り返しのみから成るものは少なく、通常、様々な種類の硫酸化、あるいは非硫酸化二糖を異なる割合で含む。コンドロイチン硫酸は、酸性ムコ多糖のうちでは最も早く発見されたものである。１８８６年、

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FischerとBoedekerによって軟骨から分離され、最初はchondroit acidと命名された。その後、硫酸エステルを持つことがわかりchondroitinsulfateと呼ばれるようになった。さらに１９５１年、Meyerらによって、コンドロイチン硫酸には３種類有り（Ａ，Ｂ，Ｃ）軟骨中にはＡとＣが存在することが明らかにされた。コンドロイチン硫酸鎖を持つプロテオグリカンとしては、アグリカン、バーシカン、デコリンなどがあるが、これらの機能は不明なものも多い。しかし、アグリカンの軟骨組織機能を制御する活性や、バーシカンの抗細胞接着活性は、コンドロイチナーゼ処理により完全に消失するので、コンドロイチン硫酸鎖がそれらの活性を担っていると考えられる。さらに、網膜神経細胞に対する神経栄養因子や、神経突起伸張因子としてコンドロイチン硫酸プロテオグリカンが単離されたが、これらの活性もコンドロイチナーゼ処理により消失した。最近では、コンドロイチン硫酸由来の二糖がナチュラルキラー細胞の活性化を抑えるという報告もある。また、コンドロイチン硫酸、カラギーナン、硫酸化デキストランなどの硫酸化多糖がインフルエンザウイルスや単純ヘルペスウイルスなど多数のウイルスの細胞への感染を阻害することは、ヒト免疫不全ウイルス（ＨＩＶ）の出現以前から知られていた。このように、コンドロイチン硫酸には多様な生理活性や物性が認められることから、抗炎症剤などの医薬品をはじめ、保湿剤として化粧品あるいは目薬に、ゲル化剤・ゼリー化剤等の食品添加物として利用されており、日常生活において意外に広い範囲で見かけることができる。また、これら通常の用途以外にも、その特性から様々な目的の医薬品などとして潜在的な利用価値が期待されている。

【０００３】

【発明が解決しようとする課題】現在医療等に用いられているコンドロイチン硫酸は、鯨軟骨から抽出したコンドロイチン硫酸Ａ（ＣｈＳ－Ａ、コンドロイチン４硫酸）及び鮫の鰭から抽出したコンドロイチン硫酸Ｃ（ＣｈＳ－Ｃ、コンドロイチン６硫酸）である。最近では、捕鯨禁止によってＣｈＳ－ＡからＣｈＳ－Ｃに比重が移りつつあるが、鮫の鰭も中華料理の食材として価格が上昇している。そのため、より安価に、且つ大量にコンドロイチン硫酸を取得できる原材料及び方法が求められている。従って、本発明の目的は、より安価に、且つ大量に取得でき、尚かつ種々の有用な用途が期待できる新規なコンドロイチン硫酸と、その取得方法を提供することにある。

【０００４】

【課題を解決するための手段】本発明は、非硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンを含む二糖単位（以下、非硫酸化ＧａｌＮＡｃと略す。）：（１１．０±３．３）％、Ｃ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンを含む二糖単位（以下、Ｃ６位一硫酸化ＧａｌＮＡ

ｃと略す。）：（５２．８±１５．８）％、Ｃ４位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンを含む二糖単位（以下、Ｃ４位一硫酸化ＧａｌＮＡｃと略す。）：（２８．４±８．５）％、及びＣ４，Ｃ６位二硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンを含む二糖単位（以下、Ｃ４，Ｃ６位二硫酸化ＧａｌＮＡｃと略す。）：（７．８±２．３）％を含んで成るコンドロイチン硫酸、及び、鮭の鼻軟骨を低温粉砕し、脱脂した後、アルカリ及びプロナーゼで処理し、得られた消化液を遠心分離後エタノール沈殿させることを特徴とする該コンドロイチン硫酸の取得方法、並びに、得られた沈殿を、更に、陽イオン交換樹脂で処理して該コンドロイチン硫酸を製造する方法、に関する。また本発明は、該コンドロイチン硫酸を含んでなる抗炎症剤及び保湿剤に関する。

【０００５】本発明のコンドロイチン硫酸は、例えば鮭の鼻軟骨から大量に得られる。当該方法の概略は以下の通りである。即ち、先ず、鮭の加工工程中に排出される頭部から表皮、硬骨、肉粒などを除き、鼻軟骨のみを分離して－１２０℃で粉砕したものを原料とする。低温粉砕することで、粉砕熱の発生による物質の変性及び酸化を防ぐことができる。また、常温粉砕では不可能なサイズへの微粉砕が可能となり、粒度も揃えられる。この軟＊

非硫酸化ＧａｌＮＡｃ（％）：１１．０±３．３
Ｃ６位一硫酸化ＧａｌＮＡｃ（％）：５２．８±１５．８
Ｃ４位一硫酸化ＧａｌＮＡｃ（％）：２８．４±８．５
Ｃ４，Ｃ６位二硫酸化ＧａｌＮＡｃ（％）：７．８±２．３

であるが、好ましくは、

非硫酸化ＧａｌＮＡｃ（％）：１１．０±２．２
Ｃ６位一硫酸化ＧａｌＮＡｃ（％）：５２．８±１０．６
Ｃ４位一硫酸化ＧａｌＮＡｃ（％）：２８．４±５．７
Ｃ４，Ｃ６位二硫酸化ＧａｌＮＡｃ（％）：７．８±１．６

である。

【０００８】コンドロイチン硫酸を構成している二糖の存在比がこのような割合のものはこれまでに知られていない。因みに、従来から知られている鯨軟骨由来のコンドロイチン硫酸では、Ｃ４位一硫酸化ＧａｌＮＡｃが約７０％と多い反面、Ｃ４，Ｃ６位二硫酸化ＧａｌＮＡｃは１％以下と少なく、同じく従来から知られている鮫軟骨由来のコンドロイチン硫酸ではＣ６位一硫酸化ＧａｌＮＡｃが約７０％と多いが、Ｃ４位一硫酸化ＧａｌＮＡｃは１０数％と少ない。

【０００９】また、本発明のコンドロイチン硫酸は硫酸基分布が従来のものよりもランダムな構造となっている。即ち、非硫酸化ＧａｌＮＡｃを含む二糖単位、Ｃ６位一硫酸化ＧａｌＮＡｃを含む二糖単位、Ｃ４位一硫酸化ＧａｌＮＡｃを含む二糖単位、及びＣ４，Ｃ６位二硫酸化ＧａｌＮＡｃを含む二糖単位がランダムに並んだ構造を有する。

【００１０】本発明のコンドロイチン硫酸は、従来のものと同様、抗炎症剤などの医薬品をはじめ、保湿剤として化粧品あるいは目薬に、また、ゲル化剤・ゼリー化剤等の食品添加物として利用しうる。更に、今後の展開として、コンドロイチン硫酸は生理的粘性が高いため配合薬剤とのブレンドによりその薬剤の局所滞留時間を延長させる効果が考えられる。また角膜コラーゲン繊維の安定化を促進し、目の組織の機能保持に有効であることが報告されていることから、鮭皮・牛皮などから抽出されるコラーゲンとのブレンドによって高機能性代用皮膚としての応用も考えられる。さらに生理・薬理活性だけではなく、高分子電解質としての特性を示すことから工業的応用も可能である。本発明のコンドロイチン硫酸は、鯨由来のコンドロイチン硫酸と鮫由来のコンドロイチン硫酸との中間的な構造を取るため、比較的広い範囲に応用できる可能性が期待できる。

【００１１】

【実施例】以下に、実施例を挙げて本発明を更に詳細に説明するが、本発明はこれらの実施例により何ら限定されるものではない。

【００１２】実施例１

＊骨粉から酸性ムコ多糖を抽出し、これをプロトン交換樹脂で処理してコンドロイチン硫酸画分を得る。操作方法等は、鯨及び鮫軟骨からのコンドロイチン硫酸抽出法のそれを参考にすることができる。

【０００６】軟骨粉から精製コンドロイチン硫酸を製造するまでを更に詳しく記すと大略以下のようになる。即ち、先ず、軟骨粉をアセトン等の有機溶剤により脱脂し、次いで、これを例えば苛性ソーダ水溶液等のアルカリ水溶液で処理し、中和した後、例えばアクチナーゼＥ等のプロナーゼで消化する。次に、この消化液を遠心分離し、酢酸等でｐＨを酸性にした後、エタノールを加えて沈殿を生じさせる。生じた沈殿を遠心分離操作で分取し、エタノールで洗浄した後、これを減圧乾燥する。得られた酸性ムコ多糖を少量の脱イオン水に溶解し、これを、例えばＤＯＷＥＸ５０ＷＸ２等の陽イオン交換樹脂で処理した後、流出液を中和し、これを脱イオン水中で透析する。得られた溶液を濃縮し、メンブランフィルター等で濾過した後凍結乾燥すれば、コンドロイチン硫酸の精製品が得られる。

【０００７】本発明のコンドロイチン硫酸を構成している二糖の存在比は、通常、

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（１）軟骨の脱脂
鮭の加工工程中に排出される頭部から表皮、硬骨、肉粒などを除き、鼻軟骨のみを分離して、液体窒素下－１２０℃で粉砕したものを原料とした。
１）約１００ｍｇの軟骨粉を２０００ｍｌの三角フラスコに入れ、アセトン７００ｍｌを加え、１０分間撹拌した。
２）５分間放置し上澄み液を除去した。
３）１）－２）の操作をさらに３回繰り返した。
４）残った沈殿を減圧デシケーターで乾燥した。
５）得られた試料は－３０℃で保存した。
（２）アルカリ処理
１）脱脂済軟骨粉５ｇを０．２Ｍ　ＮａＯＨ８０ｍｌに溶解した。
２）３７℃の湯浴中で、３時間撹拌した。
３）酢酸でｐＨ７．０に中和した。
（３）プロナーゼ消化
１）０．２Ｍ　Ｔｒｉｓ－ＨＣｌ緩衝液（ｐＨ７．８）１０ｍｌを加えた。
２）酢酸カルシウムを終濃度０．０２Ｍになるように加えた。
３）防腐のためメタノール５ｍｌを加えた。
４）アクチナーゼＥ５０ｍｇを加えた。
５）３７℃の湯浴中で、４８時間ゆっくりと撹拌した。
【００１３】（４）エタノール沈殿
１）消化液を１０，０００ｒｐｍ×３０分間、４℃にて遠心分離した。
２）上澄を０．４５μｍメンブランフィルターを用いて吸引濾過した。
３）濾過液に５％相当の酢酸カルシウムを加えた。
４）酢酸でｐＨ４．５に調整した。
５）２倍量のエタノールに加えて、４８時間放置した。
（５）沈殿の洗浄・乾燥
１）エタノール液を７，０００ｒｐｍ×３０分間、４℃にて遠心分離した。
２）沈殿を回収し８０％エタノール３００ｍｌを加え、１２時間ゆっくり撹拌した。
３）１０，０００ｒｐｍ×３０分間、４℃にて遠心分離した。
４）２）－３）の操作を繰り返した。
５）沈殿に１００％エタノール２００ｍｌを加え、６時間ゆっくり撹拌した。
６）１０，０００ｒｐｍ×３０分間、４℃にて遠心分離した。
７）得られた沈殿（酸性ムコ多糖）を減圧デシケーターで乾燥した。（脱脂済軟骨粉からの見掛歩留：４４．０％）
【００１４】（６）コンドロイチン硫酸の精製
（６－１）前処理
１）ＤＯＷＥＸ　５０ＷＸ２陽イオン交換樹脂１００ｍｌを、３Ｎ　ＨＣｌ中で２時間撹拌し、水洗後、２Ｎ　ＮａＯＨ中で２時間撹拌した。
２）上記の操作を３回繰り返した後、水洗した。
３）２．５×４０ｃｍのカラムの下に脱脂綿を詰め、空気が入らないように樹脂を詰めた。
（６－２）ＤＯＷＥＸ　５０Ｗ×２陽イオン交換樹脂処理
１）上記（５）で得られた得られた酸性ムコ多糖を、ごく少量の脱イオン水に溶解した。
２）カラムに１）を流し、２０分間放置した。
３）カラムに４００ｍｌ（樹脂体積の約４倍）の脱イオン水を流した。
４）流出液をすぐに１Ｎ　ＮａＯＨで中和した。
（６－３）精製
１）中和液を脱イオン水中で３日間透析した。
２）エバポレーターにて２０ｍｌ程に濃縮した。
３）０．２２ｍメンブランフィルターにより濾過後、凍結乾燥し乾燥標品とした。（脱脂済軟骨粉からの見掛歩留：２４．０％）
【００１５】［分析結果］上で得られた本発明のコンドロイチン硫酸（以下、ＣｈＳ－Ｓと略記する。）に関する分析結果及び考察を以下に示す。なお、これらの組成分析、構造解析等に当たっては、アミノ糖、ウロン酸の定量はMorgan-Elson法、Bitter-Muir法を用いた。また硫酸基の置換度はRhodizonate法、元素分析によって行った。ＣｈＳ－Ｓの分子量、純度検定はＧＰＣ、ＦＴ－ＩＲ、セルロースアセテート膜電気泳動を適宜使用した。構造解析は^{13}Ｃ－ＮＭＲ　（１００ＭＨｚ）及び^{1}Ｈ－ＮＭＲ（４００ＭＨｚ）を使用した。また硫酸置換位置の決定、その分布はＣｈＳ－Ｓのコンドロイチナーゼ ＡＢＣ、コンドロイチナーゼＡＣ２による二段階脱離酵素分解によって得られる不飽和二糖のＨＰＬＣ、二次元ＮＭＲ（ＣＯＳＹ^{1}Ｈ－ＮＭＲ水素核シフト相関など）分析により行った。対照サンプルとしては市販のコンドロイチン４－硫酸（ＣｈＳ－Ａ、鯨軟骨由来）及びコンドロイチン６－硫酸（ＣｈＳ－Ｃ、鮫軟骨由来）（何れも生化学工業（株）製）を使用した。
【００１６】（１）ウロン酸の定量
各試料溶液に含まれるウロン酸の割合及びこの値から求めたコンドロイチン硫酸含量（純度）を表１に、また、陽イオン交換樹脂（ＤＯＷＥＸ　５０ＷＸ２）による処理（以下、プロトン交換処理と略す。）が純度及び収率に与える影響を表２にそれぞれ示した。なお、標準コンドロイチン硫酸のウロン酸含量は３７％である。これらの結果から、本法により非常に高純度のコンドロイチン硫酸を得られることがわかった。また、プロトン交換処理前後で純度が大幅に増加している。このことからプロトン交換処理によって、ヒアルロン酸やデルマタン硫酸などの他の酸性ムコ多糖は完全に除去できたものと考えられる。

【００１７】　＊　＊【表１】

ウロン酸含量及び純度

	精製前ＣｈＳ－Ｓ	ＣｈＳ－Ｓ	ＣｈＳ－Ａ	ＣｈＳ－Ｃ
ウロン酸（％）	２５．５０	３６．４９	３５．６３	３６．８８
純度（％）	６８．９２	９８．６２	９６．３０	９９．６８

【００１８】　※　※【表２】

各操作毎の本発明のコンドロイチン硫酸の収率

	見掛歩留（％）	純度（％）	収率（％）
脱脂軟骨	１００	・・・	・・・
エタノール沈殿	４４．０	６８．９	３０．３
プロトン交換処理	２４．０	９８．６	２３．７

【００１９】（２）アミノ糖分析
各試料溶液のＮ－アセチルガラクトサミン（ＧａｌＮＡｃ）含量を表３に示した。また、プロトン交換処理前の酸性ムコ多糖からはＮ－アセチルグルコサミンが約０．３％検出されたが、ＣｈＳ－Ｓ、ＣｈＳ－Ａ及びＣｈＳ－Ｃには全く確認されなかった。その他のアミノ糖は何★れにも含まれていなかった。このことから、コンドロイチン硫酸を構成するアミノ糖はＮ－アセチルガラクトサミンのみであること、プロトン交換処理によって他のアミノ糖を完全に除去できたことが確認できた。

【００２０】　【表３】

コンドロイチン硫酸のＮ－アセチルガラクトサミン含量

	精製前ＣｈＳ－Ｓ	ＣｈＳ－Ｓ	ＣｈＳ－Ａ	ＣｈＳ－Ｃ
GalNAc（％）	２５．４６	３２．６２	３０．７０	２６．７３

【００２１】（３）元素分析
各サンプルに含まれる炭素、水素、窒素、酸素及び硫黄の重量比と硫酸化度をそれぞれ表４、表５に示した。表４からは、いずれのサンプルも同様の組成を示すことが確かめられた。表５からは、硫酸化度は鮫軟骨由来コン☆ドロイチン硫酸が最も高く、本発明のコンドロイチン硫酸は他に比べるとやや低いという結果が得られた。

【００２２】　【表４】

コンドロイチン硫酸の組成分析結果

	Ｃ（％）	Ｈ（％）	Ｎ（％）	Ｏ（％）	Ｓ（％）
ＣｈＳ－Ｓ	３９．８６	５．４８	３．２５	４５．９１	５．５０
ＣｈＳ－Ａ	３９．９６	５．４５	３．３０	４５．４０	５．８９
ＣｈＳ－Ｃ	４０．９７	５．６２	３．１５	４３．９８	６．２８

【００２３】
【表５】

コンドロイチン硫酸１分子当たりの硫酸基含量

ＣｈＳ－Ｓ	ＣｈＳ－Ａ	ＣｈＳ－Ｃ
０．７２	０．７７	０．８０

【００２４】（４）硫酸基定量
各サンプルに含まれる硫酸基及びここから算出した硫黄の比率を表６に示した。この結果から、硫酸基含量はＣｈＳ－Ｃがやや多く、ＣｈＳ－ＳとＣｈＳ－Ａは殆ど同じであることがわかった。また、これは上記（３）で述べた元素分析の結果とも一致した。

【００２５】
【表６】

各コンドロイチン硫酸の硫酸及び硫黄含量

	硫酸基（％）	硫黄（％）
ＣｈＳ－Ｓ	１８．６２	６．１５
ＣｈＳ－Ａ	１８．５２	６．１５
ＣｈＳ－Ｃ	２１．７４	７．１７

【００２６】（５）分子量測定
各サンプルの平均分子量と分子量分布を表７に示した。本発明のコンドロイチン硫酸の平均分子量は１７３，０００であった。この値は他に比べるとやや大きいが、分子量分布はほぼ一致している。一般にコンドロイチン硫酸の分子量は、抽出方法により５０，０００－３００，０００の値をとることが知られている。今回得られたコンドロイチン硫酸の分子量分布も、これに一致するものであった。

【００２７】

＊　＊【表７】

コンドロイチン硫酸の平均分子量及び分子量分布

	ＣｈＳ－Ｓ	ＣｈＳ－Ａ	ＣｈＳ－Ｃ
平均分子量	173,000	151,000	108,000
分子量分布	5-30×10⁴	5-30×10⁴	5-30×10⁴

【００２８】（６）ＨＰＬＣ

二種類のコンドロイチン分解酵素を併用して酵素分解したコンドロイチン硫酸をＨＰＬＣを用いて分画し、定量して得られた、各々のコンドロイチン硫酸を構成している二糖の存在比を表８に示す。また、ここから求めたコンドロイチン硫酸の硫酸化度（ＧａｌＮＡｃ１分子あたりの硫酸基の数）を表９に示した。表８から、本発明の※

※コンドロイチン硫酸は、鯨由来のコンドロイチン硫酸と鮫由来のコンドロイチン硫酸との中間的な構造を取ることが判った。それ故、本発明のコンドロイチン硫酸は、比較的広い範囲に応用できるのではないかと考えられる。

【００２９】

【表８】

コンドロイチン硫酸の構成不飽和二糖

	ΔＤｉ-０Ｓ	ΔＤｉ-６Ｓ	ΔＤｉ-４Ｓ	ΔＤｉ-ｄｉ４,６Ｓ
ＣｈＳ-Ｓ（％）	１１．０	５２．８	２８．４	７．８
ＣｈＳ-Ａ（％）	３．６	２５．３	７０．２	０．６
ＣｈＳ-Ｃ（％）	７．７	７１．２	１３．２	７．６

ΔＤｉ-０Ｓ　：非硫酸化ＧａｌＮＡｃ

ΔＤｉ-６Ｓ　：Ｃ６位一硫酸化ＧａｌＮＡｃ

ΔＤｉ-６Ｓ　：Ｃ４位一硫酸化ＧａｌＮＡｃ

ΔＤｉ-ｄｉ４,６Ｓ：Ｃ４,Ｃ６位二硫酸化ＧａｌＮＡｃ

【００３０】

【表９】

各々のコンドロイチン硫酸の硫酸化度

	ＣｈＳ-Ｓ	ＣｈＳ-Ａ	ＣｈＳ-Ｃ
硫酸化度	0.968	0.973	1.002

【００３１】

【発明の効果】本発明のコンドロイチン硫酸は、従来から知られている鯨由来のコンドロイチン硫酸と鮫由来のコンドロイチン硫酸との中間的な構造を取るため、比較的広い範囲に応用できる可能性が高く、従来のものと同様、抗炎症剤などの医薬品をはじめ、保湿剤として化粧★品あるいは目薬に、また、ゲル化剤・ゼリー化剤等の食品添加物として利用しうることはもとより、平均分子量が従来のものよりも大きいことから、従来のものよりも生理的粘性が高いと考えられ、配合薬剤とのブレンドによりその薬剤の局所滞留時間を延長させる効果が期待できる。また、コンドロイチン硫酸に関する最近の報告として、角膜コラーゲン繊維の安定化を促進し、目の組織の機能保持に有効である旨の報告もあることから、鮭皮・牛皮などから抽出されるコラーゲンとのブレンドによって高機能性代用皮膚としての応用も期待出来る。さらに生理・薬理活性だけではなく、高分子電解質としての特性を示すことから工業的応用も期待できる。

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